The type I insulin-like growth factor receptor pathway: a key player in cancer therapeutic resistance

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1. ABSTRACT

The insulin-like growth factor (IGF) ligands stimulate cellular proliferation and survival by activating the type I insulin-like growth factor receptor (IGF-IR). As a result, the IGF signaling system is implicated in a number of cancers, including those of the breast, prostate, and lung. In addition to mitogenic and anti-apoptotic roles that may directly influence tumor development, IGF-IR also appears to be a critical determinant of response to numerous cancer therapies. This review describes the role of the IGF-IR pathway in mediating resistance to both general cytotoxic therapies, such as radiation and chemotherapy, and targeted therapies, such as tamoxifen and trastuzumab. It concludes with a description of approaches to target IGF-IR and argues that inhibition of IGF signaling, in conjunction with standard therapies, may enhance the response of cancer cells to multiple modalities.

2. INTRODUCTION

The treatment of cancer has evolved significantly over the last decade. As our knowledge of the molecular characteristics of cancer cells advances, treatments with general cytotoxic agents and radiation are being complemented by more specific targeted therapies. For example, estrogen receptor alpha (ER) and the epidermal growth factor receptor (EGFR) family member HER-2 have emerged as outstanding therapeutic targets in breast cancer. These receptors can be specifically targeted with small molecules or, in the case of a cell surface receptor such as HER-2, with antibodies. This specific targeting of tumors helps limit some of the adverse side effects seen with general chemotherapy and radiation. Even with the increase in specificity of modern therapies, resistance to treatment is still a major problem.
Resistance to anticancer therapies can either arise de novo (intrinsically resistant at the start of treatment) or may be acquired after extended treatment. The overriding idea behind acquired resistance is grounded in the vast genetic heterogeneity of tumors. Each cell of a tumor may carry unique mutations in oncogenes and tumor suppressor genes. Thus, while a majority of cancer cells can respond to conventional therapy, there may be a small population of cells within the tumor that, due to differences in genetic composition, fail to respond. Treatment with anticancer therapies effectively selects for these resistant cells within the tumor mass, and this initially small fraction can grow to comprise the bulk of the tumor.

The insulin-like growth factor (IGF) signaling system regulates multiple levels of tumor progression, including proliferation, evasion of apoptosis, metastasis, and invasion (reviewed in Ref. (1)). Moreover, work by our lab and others has recently shown a role for the IGF system in cellular transformation both in vitro and in vivo (2-4). In addition to affecting the course of disease by promoting tumorigenesis, the IGF axis can influence clinical outcome by providing escape mechanisms from conventional cancer therapies. In fact, active signaling through the type I insulin-like growth factor receptor (IGF-IR) has been implicated in resistance to both general cytotoxic treatment regimens and to targeted therapies. This review will primarily focus on the role of the IGF axis in resistance to standard therapies, including endocrine-related therapies, growth factor signaling-targeted therapies, chemotherapy, and radiation therapy. Given the importance of IGF signaling in drug resistance, the review will conclude with an examination of available methods to target the IGF axis for cancer therapy.

3. THE IGF SYSTEM

The IGF family consists of two ligands (IGF-I and IGF-II), two receptors (IGF-IR and IGF-IIIR), six high-affinity IGF binding proteins (IGFBP 1-6), and other low-affinity IGFBP-related proteins (IGFBP-7p). The IGF ligands, formerly known as somatomedins, are 62% homologous in amino acid sequence (5) and act in endocrine, autocrine, and paracrine manners. IGF-I and IGF-II have approximately equal affinities for IGF-IR in most systems, and IGF-IR is thought to transduce the effects of both ligands. IGF-IR can also exist in a hybrid form with the structurally related insulin receptor (InsR). IGF-IR and InsR share approximately 60% amino acid sequence homology over the length of the entire protein; the kinase domains, in particular, show 84% similarity at the amino acid level. These hybrid receptors are capable of binding both IGF-I and insulin but are believed to preferentially support IGF-I signaling (6, 7). IGF-IR, a single-chain polypeptide (8, 9) that is identical to the mannose-6-phosphate receptor, only binds IGF-II. In contrast to IGF-IR, IGF-IIIR has no tyrosine kinase activity, and its role in IGF signaling remains unclear. The IGFBPs provide another level of control of IGF action by modulating the bioavailability and, thus, the physiological activities of the IGF ligands (10). There are six related IGFBPs, which are secreted proteins that can either enhance or inhibit IGF action. IGFBPs may also have IGF-independent effects on cellular processes.

4. SIGNAL TRANSDUCTION BY IGF-IR

IGF-IR is a type 2 tyrosine kinase receptor that is normally found as a heterotetramer consisting of two alpha- and two beta-subunits (11) with several alpha-alpha and alpha-beta disulfide bridges (12). Unlike other classical tyrosine kinase receptors, such as epidermal growth factor receptor and platelet-derived growth factor receptor, IGF-IR activity is dependent upon ligand-binding. Thus, overexpression of IGF-IR alone does not result in receptor activation (13). Ligand-binding induces a conformational change in receptor subunits, resulting in activation of the intrinsic tyrosine kinase of the cytoplasmic domain of IGF-IR (reviewed in (14)). The kinase both autophosphorylates and transphosphorylates the receptor (15), resulting in the phosphorylation of adaptor proteins. Thus, protein complexes that transduce the intracellular signal are assembled. Although various cytoplasmic proteins, including SHC (16), GAB (17), and CRK (18), can interact with activated IGF-IR, it is principally the insulin receptor substrate (IRS) family of adaptor proteins that is responsible for mediating signals downstream of IGF-IR (reviewed in (19)). IGF-IR-mediated phosphorylation of both IRS-1 and SHC, coupled with Grb-2/Sos interaction (20), activates the Ras/mitogen-activated protein kinase (MAPK) cascade, which stimulates cell growth and proliferation. Conversely, IGF-IR-mediated protection from apoptosis results from activation of phosphatidylinositol 3'-kinase (PI3K) and Akt (21). One major mechanism by which PI3K/Akt signaling promotes cell survival is through phosphorylation and subsequent inactivation of the pro-apoptotic protein BAD (22, 23). IGF signaling has also been shown to promote cancer cell motility, and this is thought to be largely dependent on phosphorylation of IRS-2 and integrin expression (24). The signaling pathway downstream of IRS-2 responsible for IGF-IR-mediated cell motility is not clearly defined but seems to involve the small G-protein RhoA and multiple kinases, including focal adhesion kinase (FAK), Rho-kinase (ROCK), PI3K, and the MAPKs p38 and ERK1/2 (25).

5. IGF SYSTEM IN RESISTANCE TO ENDOCRINE THERAPIES IN BREAST CANCER

Approximately 70% of breast cancers express ER (26), which regulates expression of genes involved in tumor initiation and progression. Thus, for these hormone-responsive tumors, ER is a common therapeutic target. Multiple drugs have been used to inhibit ER function. These include selective estrogen receptor modulators (SERMs), such as tamoxifen, selective estrogen receptor down-regulators (SERDs), such as fulvestrant, and aromatase inhibitors (AIs), such as letrozole, anastrozole, and exemestane. Although each class of drug functions in a different manner to block ER activity, all three therapies are subject to resistance. Thus, despite significant advances in breast cancer care, resistance to endocrine therapy remains a major problem.
The type IGF-IR pathway: a key player in cancer therapeutic resistance?

One driving force behind the failure to respond to endocrine therapy is molecular cross-talk between ER and various growth factor signaling pathways, including the IGF system. Components of the IGF system can interact with the ER pathway at many levels, and this interaction is bidirectional. ER can increase expression of several IGF signaling components, including IGF-II (27), IGF-IR (28), and IRS-1 (29). While these studies have shown that estrogen action can enhance IGF signaling components, other work has shown that IGFs can also enhance ER activity. IGF-1 can directly activate ER in a ligand-independent manner in cell line models (30) and in vivo (31). Furthermore, ER is likely to physically interact with components of the IGF signaling cascade, including IGF-IR (32), PI3K (33), IRS-1 (34), and Shc (35).

It is widely accepted that aberrant activation of growth factor signal cascades can promote anti-hormone failure in breast cancer cells. Given the extensive molecular cross-talk that occurs between ER and IGF signaling pathways, it is not surprising that the IGF system has emerged as an important player in resistance to endocrine therapy in breast cancer.

5.1. Resistance to tamoxifen treatment

Tamoxifen is classified as a SERM and has been the most widely used form of endocrine therapy for the past 30 years. Tamoxifen binds ER in place of estradiol and alters the molecular conformation of the receptor (36). This conformational change results in preferential recruitment of corepressors to ER and attenuation of receptor transcriptional activity (37). While the overall effectiveness of tamoxifen to treat both early and advanced breast cancer has been quite good, approximately 40% of ER-positive patients fail to respond to treatment (38). While much work has focused on the roles of EGFR and HER-2 in tamoxifen resistance (reviewed in (39)), emerging data have emphasized the importance of IGF-IR signaling in this process as well.

To date, a majority of the data concerning the IGF network in resistance to endocrine therapy comes from cell line models. While most work has been conducted in MCF-7 breast cancer cells because they express high levels of IGF-IR and ER, and are quite responsive to IGFs, some evidence for resistance to tamoxifen also comes from the IGF-responsive T-47D breast cancer cell line. Given the extensive cross-talk that occurs between IGF-IR and ER pathways, it is not surprising that treatment of MCF-7 cells with tamoxifen severely attenuates both ER and IGF-IR signaling during the growth-inhibitory phase (40). While this initial response is characterized by reduced IGF-IR signaling capacity, prolonged exposure to tamoxifen eventually leads to acquired drug-resistant growth that occurs with evidence of re-established growth factor signaling. While this resistant phenotype has been primarily associated with increased expression of both EGFR (41, 42) and HER-2 (41), reactivation of IGF-IR signaling also appears to be critical. Under tamoxifen-resistant conditions, ER acquires the ability to stimulate expression of IGF-II, a potent ligand of IGF-IR (43). As a stimulator of cell proliferation and survival, IGF-IR signaling promotes the drug-resistant phenotype in multiple ways. First, IGF-IR has been shown to phosphorylate and increase activity of EGFR in a c-src-dependent manner (41). This cross-talk between IGF-IR and EGFR is not unique to MCF-7 cells, as it has also been shown to occur in a tamoxifen-resistant T-47D cell line (43). Second, IGF-IR couples to the PI3K/Akt pathway, which can phosphorylate ER on a conserved serine residue (Ser-167) located within the activation function-1 region of the receptor (44). This phosphorylation event is significant since it can abrogate the antagonistic action of tamoxifen, in part by protecting breast cancer cells from tamoxifen-induced apoptosis (44).

The importance of IGF signaling in resistance to anti-hormonal therapy has also been shown in studies using inhibitors of IGF action. Parisot et al. showed that growth of the tamoxifen-resistant cell line MCF-7/5-23 (subclone of the MCF-7 cell line) can be blocked by treatment with alpha-IR-3, a monoclonal antibody directed against IGF-IR (45). In a similar fashion, another group showed that AG1024, an inhibitor of IGF-IR tyrosine kinase activity, can inhibit growth of tamoxifen-resistant MCF-7 cells (46). Data obtained from these inhibitor studies and from the previously mentioned signal transduction experiments clearly show that IGF action plays a significant role in endocrine insensitivity in vitro.

Compared to the cell culture data, much less is known about the role of IGF signaling in tamoxifen resistance in clinical breast cancer. Recent immunohistochemical analyses have been performed on a small cohort of tumors from the Nottingham historical, paraffin-embedded, primary breast cancer series. These samples were taken from patients who have ER-positive/EGFR-positive acquired tamoxifen resistance. Using antibodies against both total IGF-IR and phosphorylated IGF-IR, Gee et al. found that IGF-IR was expressed and active in these samples (38). Although these data only represent an association between tamoxifen resistance and IGF-IR activity and not a causal relationship, it is possible that IGF signaling is important for endocrine insensitivity in vivo. It is clear that more work needs to be done to understand the role of the IGF system in tamoxifen resistance in clinical breast cancer, and an analysis of IGF-IR expression and activity at the time of acquired resistance might be especially informative. It might also be critical to analyze expression and phosphorylation status of downstream adaptor proteins, particularly IRS-1 and IRS-2, as these molecules may be rate-limiting for IGF-IR action. Indeed, several studies have shown the importance of these adaptor proteins in mediating tumorigenesis both in vitro and in vivo (4, 47).

5.2. Resistance to raloxifene treatment

Raloxifene, a second generation SERM, is approved for the prevention and treatment of osteoporosis in postmenopausal women (48). Similar to tamoxifen, raloxifene acts as an estrogen antagonist in the breast, and several clinical trials have suggested that raloxifene may play a role in minimizing the risk of invasive breast cancer in postmenopausal women (49-51).
There is not much published data available concerning the role of IGF-IR in resistance to raloxifene. However, since raloxifene and tamoxifen exhibit similar mechanisms of action and since the two drugs may display cross-resistance, the development of insensitivity to raloxifene may be similar to that of tamoxifen. For example, O’Regan et al. developed a raloxifene-resistant tumor model in vivo and showed that these tumors display increased expression of both EGFR and HER-2 (52). This increase in epidermal growth factor family member expression is similar to what occurs in tamoxifen-resistant cells (41). Although IGF signaling is re-established in the tamoxifen-resistant model due to upregulation of IGF-II, IGF-IR activity was not examined in the raloxifene-resistant model system. It would be very interesting to determine if IGF-IR function is enhanced subsequent to raloxifene resistance and whether this plays a crucial role in maintaining the insensitivity phenotype.

5.3. Resistance to fulvestrant treatment

Fulvestrant belongs to the SERD class of ER antagonists and has shown no estrogen-agonist activity in either preclinical or clinical studies (53-56). Fulvestrant binds competitively to ER, inhibits receptor dimerization (57), and reduces the receptor’s half-life by increasing protein turnover (58). Thus, fulvestrant’s mechanism of action is distinct from that of tamoxifen, and, in fact, fulvestrant is recommended for the treatment of ER-positive metastatic breast cancer in postmenopausal women with disease progression following acquired tamoxifen resistance (59).

Loss of ER protein expression resulting from fulvestrant treatment severely attenuates the ability of cancer cells to proliferate. However, as is the case for prolonged treatment with a SERM such as tamoxifen, extensive treatment with fulvestrant can also result in resistance. The cells begin to rely on other, ER-independent pathways for maintaining their tumorigenic character. Growth factor signaling has emerged as a prime candidate for mediating this alternate pathway. Data from one study showed that MCF-7 cells that are significantly growth inhibited by fulvestrant may be stimulated to proliferate in the presence of this agent by exogenously administered IGF-I (60). The fact that these cells could proliferate in response to IGF-I stimulation despite severe reduction in ER protein levels suggests that IGF-IR signaling may provide an alternate pathway for cell growth upon fulvestrant resistance. These findings are supported by another group that developed a fulvestrant-resistant cell line model by culturing MCF-7 cells in hormone-free medium supplemented with fulvestrant (10^{-7} mol/L) for 12 months (61). These fulvestrant-resistant cells could undergo a slight increase in growth rate upon stimulation with IGF-I (100 ng/mL).

As previously stated, particularly in the case of insensitivity to tamoxifen, IGF-IR signaling can enhance ER phosphorylation and activity. However, a reduction in ER expression is also possible when aberrant and sustained growth factor signaling occurs (60). Thus, loss of ER in these instances of extreme growth factor signal transduction may lead to complete endocrine insensitivity. For example, treatment of MCF-7 cells with IGF-I resulted in a significant decrease in ER protein expression over the 24-hour time course that was monitored (62). Other work has revealed that a decrease in levels of ER is largely dependent on active PI3K signaling (63), hyperactive MAPK (extracellular signal-regulated kinases, ERK1/2), and elevated activity of nuclear factor-kappa-B (NF-xB) (64). While a direct association between PI3K function, hyperactive ERK1/2, an increase in NF-xB activity, and IGF-IR signaling was not made in either of these studies, it is feasible that IGF signal transduction plays a role in loss of ER in these systems. PI3K and ERK1/2 signal transduction cascades are well-characterized downstream arms of the IGF-IR pathway, and our lab has recently shown that a constitutively active IGF-IR variant can lead to increased NF-xB activity (3). Although there is still much that needs to be learned concerning ER down-regulation by growth factor signaling, preliminary studies support this idea and suggest a mechanism for resistance to fulvestrant and to other anti-hormonal therapies.

5.4. Resistance to treatment with aromatase inhibitors

Many strategies for the treatment of hormone-dependent breast cancer have focused on targeting ER function. However, methods to deplete estrogen availability can also be used to block receptor activity. This is the mode of action of aromatase inhibitors, such as anastrozole, letrozole, and exemestane. Anastrozole and letrozole are two non-steroidal compounds that act as competitive inhibitors with respect to the androgen substrates (65). Exemestane is a steroidal derivative, which is itself chemically converted into a reactive species that can irreversibly inactivate the aromatase enzyme (65). While the mechanism of exemestane action is slightly different from the mechanism of action of both anastrozole and letrozole, all three compounds effectively inhibit the enzyme aromatase, which is responsible for converting androgen precursors into estrogens. Aromatase inhibitors are now widely used in the endocrine treatment of hormone-dependent breast cancer in postmenopausal women. However, as with all prolonged therapy, insensitivity to aromatase inhibitors does develop, and the mechanism of resistance has not been completely elucidated.

To date, most of the available data concerning resistance to aromatase inhibitors are derived from laboratory studies. Many of these studies have used long-term estrogen deprivation (LTED) to mimic the function of aromatase inhibitors in blocking estrogen biosynthesis. One group reported enhanced cross-talk between IGF-IR and ER in cells that underwent LTED (66). They emphasized the activation of both MAPK and PI3K cascades and the hypersensitive response to estrogen stimulation, potentially through non-genomic ER activity. The increased function of IGF-IR in these cells may be supported by earlier work showing that short-term anastrozole treatment increased IGF-I and IGF-II while decreasing IGFBP-1 (67).
Despite these initial findings, there is still much that is unknown concerning mechanisms of resistance to aromatase inhibitors. This is highlighted by differences in LTED cell line models. For example, while Santen et al. found enhanced IGF-IR activity in their LTED cells (as previously discussed), Martin et al. discovered an increase in HER-2 function in cells that were deprived of estrogen in a similar manner (68). Thus, there are clear differences in cell line models generated by different laboratories. Furthermore, it is important to keep in mind that while estrogen deprivation may mimic certain aspects of aromatase inhibitor function, it does not exactly replicate the loss of function of this enzyme. In this respect, aromatase inhibitor-resistant models may be better suited to study mechanisms of resistance. Finally, given that exemestane functions differently from anastrozole and letrozole, it is possible that resistance mechanisms may differ depending on the type of treatment administered. For example, it is feasible that in the case of resistance to anastrozole or letrozole, a conformational change of aromatase may lead to decreased affinity of these inhibitors for the enzyme. As for exemestane, a reduced ability to be catalytically converted into a reactive species may be the cause of resistance to this drug. To truly understand these resistance mechanisms, it is clear that much more work must be done.

6. IGF SYSTEM IN RESISTANCE TO EGFR/HER-2-TARGETED THERAPIES

The EGFR family of receptor tyrosine kinases consists of four members that mediate proliferation, differentiation, and survival of both normal and malignant epithelial cells (69, 70). To date, most cancer-related studies have specifically concentrated on the roles of EGFR and HER-2 in tumorigenesis. This focus is warranted given the overexpression of these receptors in several solid tumor types. For example, EGFR is found to be overexpressed in 40-80% of non-small cell lung cancer (NSCLC) cases (71). HER-2 is overexpressed in approximately 30% of human breast cancers and is associated with poor prognosis (72, 73). Given that these proteins are cell surface receptors that activate multiple downstream signaling cascades and that they are highly expressed in several cancers, EGFR and HER-2 have proven to be useful drug targets.

Both small molecule tyrosine kinase inhibitors and monoclonal antibodies have been used to inhibit signaling through EGFR family member proteins. The small molecule inhibitor gefitinib (Iressa) blocks signaling through EGFR by hindering the ATP-binding pocket of the receptor (74). Trastuzumab (Herceptin) is a humanized monoclonal antibody directed against an extracellular portion of HER-2 and is approved for the treatment of breast cancers that overexpress HER-2 (75). Despite the initial success of these drugs in cancer treatment, resistance to both gefitinib and trastuzumab eventually develops. Several mechanisms of resistance have been proposed but particular attention has been paid to the IGF system in this process.

Examination of IGF-IR in resistance to therapies targeted against EGFR and HER-2 is warranted given the available data highlighting interplay between these two systems. For example, EGF ligand can induce transformation of wild-type mouse embryonic fibroblasts (MEFs) overexpressing EGFR (76). However, EGF fails to induce transformation of EGFR-overexpressing MEFs derived from littermate embryos in which the Igf1r gene has been disrupted. Thus, this study suggests that EGFR requires IGF-IR for its transforming ability. Interaction between IGF-IR and HER-2 has also been found, and it appears that, at least in breast tumor cells, IGF-IR directs HER-2 phosphorylation (77). These data, elucidating an important role for IGF-IR in both EGFR and HER-2 signaling capacity, clearly support the idea that the IGF system likely influences response to both EGFR- and HER-2-targeted therapies.

6.1. Resistance to gefitinib treatment

Gefitinib is a small molecule tyrosine kinase inhibitor of EGFR that inhibits proliferation of several human cancer cell types, including NSCLC and breast cancer (78). Clinical trials with gefitinib have been conducted and, while promising, it is clear that a significant percentage of patients do not respond to treatment. Furthermore, even responders may exhibit disease progression within a few months of therapy. Thus, both de novo and acquired resistance pose a problem for treatment. Chronic blockade of EGFR signaling by a compound such as gefitinib may result in tumors that become more dependent on other growth factor signaling pathways. EGFR and IGF-IR are often coexpressed in human cancers (79, 80), and data suggest that IGF-IR plays a role in resistance to gefitinib in a number of cancer cell types.

\textit{In vitro} data generated in human breast cancer cell lines have revealed a role for IGF-IR in gefitinib insensitivity. Using EGFR-positive MCF-7-derived tamoxifen-resistant breast cancer cells (TAM-R), Jones et al. generated a gefitinib-resistant subline (TAM/TKI-R) after 6 months of exposure to the tyrosine kinase inhibitor (81). The growth-inhibitory effects of gefitinib on the TAM-R cells could be substantially prevented by exposing the cells to IGF-II (43). Furthermore, the TAM/TKI-R cells did not exhibit basal phosphorylated EGFR. However, compared to the parental cells, the gefitinib-resistant cells displayed increased levels of activated IGF-IR and were also more sensitive to growth inhibition by AG1024, a tyrosine kinase inhibitor of IGF-IR. Thus, it appears that the gefitinib-resistant cells are more dependent on IGF-IR signaling than their parental counterparts. Chronic blockade of EGFR signaling activity by gefitinib may promote dependence on other growth factor pathways, such as IGF-IR, in the resistant model. Like the experiments performed with these MCF-7 derivatives, work in other breast cancer cell lines has also supported a role for IGF-IR in gefitinib resistance. For example, overexpression of IGF-IR in SKBR3 cells caused a significant increase in gefitinib insensitivity (79). In addition, combined administration of gefitinib and AG1024 cooperatively reduced proliferation in all cell lines tested, including MCF-7, SKBR3, MDA-MB-468, and MDA-MB-231 (79).
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The IGF system seems to be an important player in gefitinib resistance in lung cancer cells as well. A recent preclinical study showed that gefitinib could induce apoptosis in NSCLC cells when IGF-IR signaling was suppressed (82). However, administration of gefitinib alone had only minor effects on apoptosis. Furthermore, treatment with gefitinib resulted in heterodimerization between EGFR and IGF-IR and subsequent activation of IGF-IR signaling. Thus, NSCLC cells that experience blockage of EGFR signaling due to prolonged exposure to gefitinib may become more dependent on signaling through IGF-IR. Although these preclinical data suggest that IGF-IR may play a role in the development of resistance to gefitinib, data from another study examining gefitinib-treated patients with advanced NSCLC delivered unexpected results. Immunohistochemical and statistical analyses revealed that expression of IGF-IR was not correlated with response to gefitinib treatment (83). This does not completely contradict the preclinical data. Since mere expression of IGF-IR does not seem to correlate with gefitinib resistance, other biomarkers, such as IGF-IR phosphorylation status or expression of IRS-1 or IRS-2, may be more appropriate to predict response to gefitinib treatment.

Erlotinib (Tarceva) is another small molecule inhibitor of EGFR that functions, similarly to gefitinib, by blocking the ATP binding site of the cytoplasmic domain. Resistance to this drug is also a common occurrence and, like gefitinib, appears to involve heterodimerization between IGF-IR and EGFR. Treatment of NSCLC cells with erlotinib increased levels of IGF-IR/EGFR heterodimers and activated IGF-IR signaling (84). Therefore, the data obtained from experiments involving erlotinib, in conjunction with results from studies examining response to gefitinib, suggest that IGF-IR is an important player in resistance to several EGFR tyrosine kinase inhibitors. Furthermore, integration of IGF-IR-targeted therapies into treatment regimens for EGFR-overexpressing tumors may increase the efficacy of EGFR kinase inhibitors, such as erlotinib and gefitinib.

6.2. Resistance to trastuzumab therapy

The Her2 gene is amplified in approximately 30% of invasive breast cancers and is associated with both poor prognosis and poor disease-free survival (72, 85). As a growth factor receptor, HER-2 primarily signals through the MAPK and PI3K cascades to stimulate cellular proliferation and survival; in this manner, HER-2 contributes to tumorigenesis. Given the available data on the role of HER-2 in breast cancer, it is not surprising that this protein has emerged as a bona fide therapeutic target. In 2006, the United States Food and Drug Administration approved trastuzumab for the treatment of breast cancers overexpressing the HER-2 receptor. The mechanism of trastuzumab action is not completely defined. However, various cellular responses to trastuzumab have been observed in experimental models. Some of these responses include internalization and degradation of HER-2 (86), diminished signaling in the PI3K pathway (87), an increase in the levels of cyclin-dependent kinase inhibitor p27\(^{kip1}\) and subsequent cell cycle arrest (88), and induction of an immune response that can trigger apoptosis via antibody-dependent cellular cytotoxicity (89). According to data obtained from clinical trials, only 12-34% of patients whose tumors overexpress HER-2 respond to single-agent trastuzumab therapy (90, 91). Furthermore, in these patients who do initially respond, the median time to disease progression is approximately 5 months (91). Thus, as is the case for most drugs, patients receiving trastuzumab therapy can experience both intrinsic and acquired resistance. Combination therapies of trastuzumab in conjunction with another agent, such as chemotherapy, may help delay resistance, so understanding the mechanisms of insensitivity to trastuzumab is essential to improving treatment options for patients with breast tumors overexpressing HER-2.

There are currently several potential mechanisms of resistance to trastuzumab. However, this review will specifically focus on the role of IGF-IR in this process. Interestingly, it was the very first preclinical study examining trastuzumab insensitivity in breast cancer cells that revealed the importance of IGF-IR in resistance (92). The authors showed that SKBR3 human breast cancer cells, which overexpress HER-2 but express little IGF-IR, are quite responsive to trastuzumab, and this is evident by reduced cellular proliferation in the presence of the drug. However, when SKBR3 cells were transfected with a plasmid encoding IGF-IR (SKBR3/IGF-IR) and cultured in the presence of IGF-1, trastuzumab had no effect on proliferation. Sensitivity to trastuzumab could be restored by addition of IGFBP3, which attenuates IGF-IR signaling. A possible mechanism was revealed a few years later when the same group showed that culturing SKBR3/IGF-IR cells in the presence of IGF-1 resulted in a decrease in p27\(^{kip1}\) levels (93). The growth-inhibitory function of trastuzumab has been suggested to involve p27\(^{kip1}\) and, thus, IGF-1 may antagonize trastuzumab activity at the level of the cell cycle. In addition, other work performed in HER-2-stably transfected MCF-7 human breast cancer cells (MCF-7/HER-2), which express high levels of endogenous IGF-IR, has revealed similar results. When IGF-IR signaling was activated in MCF-7/HER-2 cells, trastuzumab had little effect on minimizing proliferation (92).

The role of IGF-IR in resistance to trastuzumab was further explored by using an SKBR3 cell line derivative that was made resistant to trastuzumab by long-term exposure to the drug (94). While levels of total IGF-IR were not changed between trastuzumab-sensitive and trastuzumab-resistant cells, a unique interaction was found to occur between IGF-IR and HER-2 solely in the trastuzumab-resistant line (95). This unique interaction in the resistant cells allows for cross-talk to occur between IGF-IR and HER-2. While IGF-1 stimulation resulted in increased HER-2 phosphorylation in the trastuzumab-resistant cells, it did not increase phosphorylation of HER-2 in the sensitive parental cells. Furthermore, blockade of IGF-IR signaling led to diminished HER-2 phosphorylation only in the resistant cells. Interestingly, the interaction between IGF-IR and HER-2 could be disrupted by addition of either an anti-IGF-IR antibody or an anti-HER-2 antibody, and this disruption of the heterodimer could significantly restore trastuzumab sensitivity.
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In addition to the vast cell culture data that has been obtained, IGF signaling seems to be an important component of trastuzumab resistance in vivo as well. Jerome et al. showed that tumor progression of advanced-stage MCF-7/HER-2 xenografts could be delayed by addition of recombinant human IGFBP3, and that treatment with this binding protein also potentiated trastuzumab activity (96). The authors found that inhibition of HER-2-overexpressing human breast tumor growth by IGFBP3 is associated with restored downregulation of Akt and p44/p42 MAPK phosphorylation in vivo, and this may explain the potentiation of trastuzumab activity.

Despite the wealth of preclinical data that suggest a role for IGF-IR in development of resistance to trastuzumab, the clinical relevance of IGF-IR in resistance is still unclear. Recently, a clinical study was performed to examine IGF-IR expression in tumor samples from patients (n = 72) receiving trastuzumab therapy for HER-2-overexpressing metastatic breast cancer (97). Immunohistochemical and statistical analyses did not reveal any correlation between IGF-IR expression and response to trastuzumab. Thus, contrary to a majority of the preclinical evidence, these clinical data seem to argue against a role for IGF-IR in resistance to trastuzumab. However, this does not imply that IGF-IR is not an important determinant for response to trastuzumab. Even though mere IGF-IR expression may not predict trastuzumab sensitivity, other factors, such as IGF-IR phosphorylation or heterodimerization between IGF-IR and HER-2, may be better predictors of response to trastuzumab treatment. Clearly, more work needs to be done to fully elucidate the role of IGF-IR in resistance and its clinical relevance.

7. IGF SYSTEM INFLUENCES RESPONSE TO RADIATION

Approximately half of all cancer patients receive some type of radiation therapy. The treatment uses ionizing radiation to kill cancer cells and shrink tumors by inducing DNA damage, which inhibits the ability of the cells to grow and proliferate. Cell signaling pathways that regulate survival and the DNA damage response are key players in modulating the outcome of radiation therapy. Indeed, IGF-IR, a well-characterized survival factor (98), has also been shown to be involved in DNA repair (99-101). Additionally, IGF-IR is activated in response to ionizing radiation (102). Thus, it may come as no surprise that the IGF system has been heavily implicated in sensitivity to radiotherapy.

Some of the first studies examining the role of IGF-IR in response to radiation were performed in MEFs lacking endogenous IGF-IR (R-) due to a null mutation in the Igfir gene (103). R- cells were much more sensitive to ionizing radiation than R+ cells (derived from R+ MEFs by stable transfection of Igfir). In fact, while many R- cells began to die only 48 hours after irradiation, a majority (~90%) of R+ cells remained viable even 4 days after irradiation exposure, suggesting that IGF-IR plays an important role in preventing radiation-induced cell death.

Studies in other cell types, including NIH 3T3 fibroblasts (104), mouse melanoma cells (105), MCF-7 human breast cancer cells (106), several human prostate cancer cell lines (107, 108), and multiple human lung cancer cells (102, 109), have supported these initial findings and reinforce a role for IGF-IR in mediating response to radiation treatment.

The data generated from these cell culture experiments seem to be supported by clinical evidence as well. For example, immunohistochemical analysis of primary breast tumors revealed that high levels of IGF-IR in tumor samples strongly correlate with ipsilateral breast tumor recurrence following lumpectomy and radiation therapy (104).

Although the exact mechanism by which IGF-IR modulates sensitivity to radiotherapy has not been completely elucidated, recent studies have made significant contributions to our understanding of this process. Mutational analysis of IGF-IR has revealed the necessity of both tyrosine-950 and the carboxyl-terminus for radioresistance (110). This suggests that signaling downstream of IGF-IR is important for mediating the effect of radiation, and the two pathways that seem to play important roles in this process are the cell survival pathway and the DNA damage response pathway.

Activation of PI3K/Akt is associated with cell survival (22), and these kinases are well-characterized downstream components of the IGF signaling system (21). Thus, one mechanism of IGF-IR-mediated radioresistance occurs through activation of PI3K/Akt and subsequent evasion of apoptosis elicited by ionizing radiation.

As previously stated, the DNA damage response pathway also appears to be important. Both ataxia-telangiectasia mutated (ATM) protein kinase and Ku DNA-binding proteins are critical players in the DNA damage response, and recent studies have linked IGF-IR to both of them. The ability of ATM kinase to coordinate the cellular response to DNA damage, which results in either cell survival or apoptosis, largely depends on its ability to regulate Igfir gene expression. Two studies have shown that cells with defective ATM kinase activity express low levels of IGF-IR and are highly radioresistant (101, 111). The interaction between these two factors appears to be bidirectional since ATM protein levels are reduced in antisense-mediated IGF-IR knockdown cells (105). Furthermore, this same study showed that while the IGF-IR knockdown cells display basal ATM kinase activity, they fail to induce further kinase activity after irradiation. This suggests that IGF-IR can modulate the function of ATM.

Another critical protein in the DNA damage response is the DNA-PK holoenzyme, which consists of both a catalytic subunit and a DNA-binding subunit. The Ku70/Ku86 protein complex is the DNA-binding component of the holoenzyme and is crucial for double-strand break repair (112). Recently, inhibition of IGF-IR signaling was shown to result in radiosensitivity, in part by downregulating expression of Ku86 (102). The radiosensitivity likely can
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Figure 1. Methods of blocking IGF signal transduction as potential cancer therapy. Multiple strategies can be used to disrupt signal transduction of the IGF system in cancer. These include reduction of circulating IGF-I levels, neutralization of IGF-I or IGF-II ligands, blockade of IGF-IR activation, or decreased expression of IGF-IR.

be explained by a cellular decision to undergo apoptosis when DNA damage cannot be repaired.

The studies performed to date clearly support a role for IGF-IR signaling in mediating the response to ionizing radiation. Thus, it is likely that patients receiving radiotherapy will benefit from co-administration of an IGF-IR inhibitor.

8. IGF SYSTEM AND RESISTANCE TO CHEMOTHERAPY

Targets of chemotherapy drugs are vast and include DNA itself, enzymes, such as topoisomerases, and structural proteins, such as tubulin. Chemotherapeutic agents have been used in the adjuvant setting to reduce the odds of recurrence and death in patients suffering from a variety of cancers. Despite the general success of chemotherapy, treatment does not escape eventual development of resistance. There are several possible reasons for chemotherapy resistance, including the failure of cancer cells to take up the chemotherapeutic agent, the ability of cancer cells to effectively pump the drug out of the cells, and the ability of cancer cells to activate survival pathways, which inhibit chemotherapy-induced apoptosis. For the purposes of this review, we will focus on this third mechanism of resistance and, specifically, the role of IGF-IR signaling in this process.

In addition to promoting mitogenic activity, IGF-I signaling through IGF-IR can protect multiple cells types from apoptosis. This has been demonstrated experimentally in a number of models. For example, fibroblasts are protected from cell death by IGF-IR (113). This same study showed that this protection depends on the kinase activity of the receptor, as cells expressing kinase-defective mutant receptors were not protected by IGF-I. In human central nervous system atypical teratoid/rhabdoid cells, transfection of IGF-IR antisense oligonucleotides enhanced sensitivity to the chemotherapeutic drugs doxorubicin and cisplatin (114). Other experiments have shown that expression of a dominant-negative IGF-IR construct in gastric cancer cell lines and xenografts can increase chemotherapy-induced apoptosis (115). Another study performed in small cell lung cancer cell lines showed that the IGF-IR kinase inhibitor NVP-ADW742 could sensitize cells to etoposide and carboplatin (116). Furthermore, in breast cancer cells, combined administration of doxorubicin and the anti-IGF-IR antibody alpha-IR3 resulted in increased cell death in IGF-I stimulated cells than with doxorubicin alone (117).

While these studies have shown that blocking IGF-IR signaling capacity can sensitize cancer cells to chemotherapy, other studies have shown that activation of IGF-IR signal transduction can attenuate chemotherapy-induced apoptosis. For example, one study showed that overexpression of IGF-IR in hepatocellular carcinoma cells conferred resistance to doxorubicin compared to control cells (118). Another study showed that treatment of HBL100 human breast cancer cells with IGF-I increased survival of these cells in the presence of 5-fluorouracil, methotrexate, and camptothecin (119). This was due specifically to inhibition of apoptosis rather than to increased proliferation. IGF-I has also been shown to rescue MCF-7 human breast cancer cells from paclitaxel-
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and doxorubicin-induced apoptosis (120). However, unlike the results obtained in HBL100 cells, data from these experiments using MCF-7 cells showed that while IGF-I inhibited apoptosis in doxorubicin-treated cells, IGF-I had no effect on apoptosis in paclitaxel-treated cells. Instead, protection from cell death in paclitaxel-treated cells resulted from increased proliferation. Thus, the mechanism of IGF-I-mediated protection may depend on the dominant IGF-IR signaling pathway activated and the mechanism of action of cytotoxic agent employed.

Although the mechanism of IGF-mediated resistance to chemotherapy has not been completely elucidated, it is clear from multiple experimental data that this signaling pathway plays an important role in the resistance process. Thus, novel therapeutic strategies employing a combination of chemotherapy and IGF-IR-targeted therapy may enhance the response of some cells to treatment.

9. METHODS OF INHIBITING IGF ACTION IN CANCER

Multiple strategies can be used to disrupt signal transduction of the IGF system in cancer. These include blockade of IGF-IR activation, reduction of circulating IGF-I levels, neutralization of IGF-I or IGF-II ligands, or decreased expression of IGF-IR (Figure 1).

Inhibition of IGF-IR activation has been primarily achieved in one of two ways—either with the use of monoclonal antibodies directed against the extracellular domain of IGF-IR or with the use of small molecule inhibitors directed against the intracellular tyrosine kinase domain of the receptor. While small molecule inhibitors tend to block both IGF-IR and insulin receptor due to the high percentage of homology between the kinase domains of these two receptors, monoclonal antibodies directed against IGF-IR appear to act more specifically. Although these anti-IGF-IR antibodies do not appear to cross-react with insulin receptor, this does not mean that insulin signaling is not at all affected upon antibody administration. In fact, Sachdev et al. showed that treatment of MCF-7 breast cancer cells with an antibody directed against IGF-IR resulted in decreased levels of insulin receptor and diminished insulin sensitivity (121). This decrease in levels of insulin receptor upon administration of anti-IGF-IR antibody did not occur in Hs578T breast cancer cells, which have very low levels of IGF-IR expression. Thus, insulin receptor in close proximity to IGF-IR, potentially in lipid rafts or in the form of hybrid receptors, seems to be endocytosed upon treatment with anti-IGF-IR antibodies. Thus, it appears that both tyrosine kinase inhibitors and monoclonal antibodies have the capability to disrupt not only IGF-IR activity but insulin receptor signaling as well.

One of the first anti-IGF-IR antibodies to be developed was alpha-IR3 (122). Administration of this antibody was shown to inhibit both in vitro growth and xenograft growth of a number of cancer cell lines (123). Although alpha-IR3 never entered clinical testing, it helped pave the way for the development of other antibodies targeting IGF-IR, several of which are currently being tested in the clinic. For example, CP-751,871, a fully humanized anti-IGF-IR antibody (124), is undergoing a phase I clinical trial for multiple myeloma, as well as phase I and phase II trials in solid tumors. Furthermore, other anti-IGF-IR antibodies, including IMC-A12 (125) and AMG-479 (126), are currently in phase I trials in solid tumors, including breast carcinoma. Although the exact mechanism of action of these antibodies is not clear, IGF-IR downregulation appears to be an important component.

Small molecule inhibitors also inhibit IGF-IR activation but do so by binding to the ATP-binding pocket of the receptor. Several tyrosine kinase inhibitors have been developed, but a majority of these inhibitors have the potentially undesirable side-effect of blocking insulin receptor signaling as well. Despite this lack of specificity, various kinase inhibitors, including picropodophyllin (127), NVP-AEW541 (128), and BMS-554417 (129) have been shown to inhibit growth of various cancer cells.

To date, strategies using both monoclonal antibodies and small molecule tyrosine kinase inhibitors to block IGF-IR activation are furthest along in terms of clinical development. However, other strategies to disrupt IGF signal transduction in cancer cells may also prove to be promising. For example, circulating levels of IGF-I can be reduced with a growth hormone antagonist, such as pegvisomant. IGF-I and IGF-II ligands can potentially be neutralized by various methods, including administration of binding proteins or treatment with antibodies directed against IGF-I or IGF-II. Finally, expression of IGF-IR itself can be diminished with either antisense oligonucleotides or through RNA interference. Although each type of strategy poses its own problems as far as clinical development is concerned, there is much promise in the field of IGF-IR inhibition.

10. CONCLUSION

Several studies implicate the IGF signaling system in regulating multiple aspects of the malignant phenotype, including resistance to both cytotoxic and targeted therapies. While this undoubtedly reflects the ability of IGF-IR to protect cells from apoptosis, such an explanation appears to be too simple. The IGF system interacts with and influences various cellular components, including ER, EGFR, HER-2, and the DNA damage response pathway. It is through these interactions that IGF-IR appears to cause resistance to multiple therapies. While data obtained from numerous cell culture and animal models support this hypothesis, the currently available clinical data is less clear. In patients, mere expression of IGF-IR may not correlate with resistance to standard therapies. Thus, other biomarkers must be identified. For example, phosphorylation status and activity of IGF-IR may be indicative of the ability of tumor cells to respond to certain therapies. Expression of proteins downstream of IGF-IR, in particular the IRSs, may also have to be considered, and gene expression profiling may help identify tumors that are sensitive or resistant to specific treatments.
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Preclinical studies have provided a wealth of information regarding the role of IGF-IR in development and maintenance of insensitivity to numerous cancer therapies. The next challenge is to translate these findings to the clinic, where they can be useful in determining a treatment regimen for patients. Of course, this largely depends on the efficiency of strategies aimed at disrupting IGF signaling. Many of these strategies are now undergoing clinical trials, and, hopefully, the results of these studies will go a long way to most effectively inhibit IGF-IR action and potentially enhance the response of tumor cells to treatment.

11. ACKNOWLEDGEMENTS

This work was supported in part by the Susan G. Komen Breast Cancer Foundation grant BCTR0601346 (X.C.) and U.S. Army Department of Defense research grant W81XWH-06-1-0714 (A.J.C.). A.J.C. is the recipient of a Rockwell Scholar award (Department of Molecular and Cellular Biology, Baylor College of Medicine). R.K.D. is a recipient of a Komen Research Foundation Postdoctoral Fellowship. A.V.L. is a recipient of a T.T. Chao Scholar award (Department of Medicine, Baylor College of Medicine).

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**Key Words:** breast cancer, chemotherapy, EGFR, endocrine therapy, estrogen receptor, HER2, IGF, IGF-IR, radiation, targeted therapy, therapeutic resistance.

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